

## Detection of polymyxin resistance: evaluation of rapid polymyxin B test and agar screening test with 3µg/mL

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### Abstract

Carbapenem-resistant Gram-negative infections are a subject of major concern in many regions of the world. For these infections, polymyxins have been extensively used, enhancing selective pressure among bacteria; therefore, resistance rates are increasing. The discovery of plasmid-mediated resistance thickens the problem. In clinical microbiology laboratories, disk diffusion or gradient-based tests are not reliable and Broth Microdilution (BMD) is recommended by CLSI and EUCAST. We evaluated a screening test, based on a homemade agar dilution plate (3 µg/mL), as well as the Rapid Polymyxin NP test searching for less time-consuming and easier to perform techniques with results as reliable as BMD. Intra-plate reproducibility in agar screening was evaluated by testing isolates in duplicate distributed in opposite points of the plate. Also, we performed agar screening with freshly prepared plates and with plated stored for seven days at 4-8 °C. Overall, 148 *Enterobacterales* were evaluated: 55 resistant and 93 susceptible to polymyxin B. Resistant isolates included four intrinsically-resistant clinical isolates, as well as four isolates harboring *mcr-1* gene. Rapid Polymyxin NP test showed 98.2%, 97.8%, 96.4% and 98.9% of sensitivity, specificity, positive and negative predictive values, respectively. Almost all (94.4%) truly positive results changed color (from red to yellow) after 2h of incubation. Sensitivity, specificity, positive and negative predictive values for agar dilution were 94.5%, 97.8%, 96.3% and 96.8%, respectively. Duplicates positioned in different points of the plate were fully concordant. Same results were observed using 7 days-storage plates, except for one *K. pneumoniae* that have grown in those plates, despite its MIC of 0.5 µg/mL. Overall, Rapid Polymyxin NP and agar dilution demonstrated to be reliable methods to detect polymyxin resistance.

Besides, preparing and storing agar plates with polymyxins seems to be a good option for clinical laboratories.

**Keywords: polymyxin B, susceptibility tests, agar dilution, Rapid Polymyxin NP test**